Morphological Analysis and Description of Two New Species of *Rhogeessa* (Chiroptera: Vespertilionidae) from the Neotropics

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Abstract

We recognize and formally name two new species within the *Rhogeessa tumida* complex based primarily on a genetic concept of species. Using genetic and morphological data we estimate species boundaries of the newly named taxa. Consistent genetic evidence in mtDNA, as well as autosomal and Y chromosome markers, indicate the presence of three distinct genetic lineages within what had been considered *R. tumida*. Morphologically, the two new species are similar, with small differences in skull proportion. Morphological distinctiveness among the members of the *R. tumida* complex is difficult to delineate and this has resulted in a historically intricate taxonomy. Based on available data, one of the new species is distributed mainly along the Pacific versant of Mexico and Central America. The second new species is distributed on the Atlantic versant of Central America in Guatemala, Honduras, and perhaps Nicaragua. These additions bring the number of species in the *R. tumida* complex to eight, the others being *R. aeneus*, *R. genowaysi*, *R. hussoni*, *R. io*, *R. tumida*, and *R. velilla*.

Key words: cryptic species, morphology, phylogenetics, *Rhogeessa tumida* complex, taxonomy

Resumen

Nosotros reconocemos y formalmente nombramos dos nuevas especies dentro del complejo *Rhogeessa tumida* basados principalmente en el concepto genético de especies. Utilizando información tanto genética como morfológica nosotros estimamos los límites específicos de los nuevos taxones. Evidencias genéticas consistentes en marcadores de ADN mitocondrial, cromosomas autosómicos y cromosoma Y indican la presencia de tres linajes genéticos distintos dentro de lo que ha sido considerado el complejo *R. tumida*. Morfológicamente, las dos nuevas especies son similares, con pequeñas diferencias en las proporciones craneales. La diferenciación morfológica entre los miembros del complejo *R. tumida* es difícil de delinear y esto ha resultado en una taxonomía históricamente complicada. Basados en los datos disponibles, una de las nuevas especies se distribuye a lo largo de la vertiente del Pacífico de México y Centroamérica. La segunda nueva especie está distribuida a lo largo de la vertiente Atlántica de Centroamérica en Guatemala, Honduras, y posiblemente Nicaragua. Estas adiciones elevan el número de especies en el complejo *R. tumida* a ocho, siendo las otras *R. aeneus*, *R. genowaysi*, *R. hussoni*, *R. io*, *R. tumida*, y *R. velilla*.

Palabras claves: complejo *Rhogeessa tumida*, especies crípticas, filogenia, morfología, taxonomía

INTRODUCTION

Determining what constitutes a species is one of the most difficult and controversial problems faced by biologists studying biodiversity. Numerous different species concepts have been proposed in the last century, each of which has received considerable attention and debate. When putative species show no morphological differences and reproductive behaviors are not known, the task of determining the proper taxonomy is even more difficult. One concept in particular, the Genetic Species Concept (GSC) (Baker and Bradley 2006), proposes a method of identifying species regardless of whether morphological or behavioral differences have evolved or have been documented to distinguish them. In the Genetic Species Concept (Baker and Bradley 2006), species are recognized as distinct when they have attained a level of genetic differences expected to produce incompatibility between the respective genomes of each.

The Genetic Species Concept is conceptually founded on genetic incompatibilities producing an isolating mechanism in genetically diverged phylogroups. The GSC is a consistent method of identifying species across various taxonomic groups with the support of a database of genetic information. Hypotheses of genetic incompatibility can be tested rigorously and independently by using multiple genetic markers or other types of studies such as breeding cycles, morphology, and ecology. This concept allows cryptic species to be identified more easily and accurately, which is important for understanding biological diversity and describing species and their geographic boundaries. There are several examples in mammals of cryptic species being described based on genetic differences in the absence of morphological variation (e.g., Hellborg et al. 2005, Baker and Bradley 2006). Additionally, genetic data can establish monophyletic lineages, and uncover a lack of gene flow and/or genetic variation between morphologically divergent groups to better establish a genetically well defined biodiversity of mammal species (Lausen et al. 2008; Larsen et al. 2010).

One group of mammals that exhibits high species diversity despite a lack of morphological differentiation is the *Rhogeessa tumida* complex (Chiroptera: Vespertilionidae). Members of the *R. tumida* species complex have undergone many taxonomic changes in the last several decades. Since LaVal's (1973) morphological study of the genus *Rhogeessa*, in which he considered all members of this complex to be a single species, five additional species have been described. Currently, the species complex, in addition to *R. tumida*, consists of *R. aeneus*, *R. genowaysi*, *R. io*, *R. velilla*, and *R. hussoni* (Baker 1984; Audet et al. 1993; Genoways and Baker 1996; Baird et al. 2008). In general, the newly described species were found to be morphologically difficult to differentiate from one another, but all differed karyotypically, with the exception of identical karyotypes shared between *R. genowaysi* and *R. velilla* (Baird et al. 2008). Baird et al. (2008, 2009) also demonstrated that all of these species, including *R. genowaysi* and *R. velilla*, were genetically distinct from one another based on nuclear and mtDNA sequence data. Whereas LaVal (1973) did not distinguish all of the current members of the *R. tumida* complex based on morphology, he did demonstrate that there was exceptional morphological variation present within what he considered to be a single species. More recent comprehensive morphological analyses have not been performed on this group following the description of additional species, so it remains unclear whether the variation found by LaVal (1973) is simply intra-specific variation, or whether the variation may correspond with genetic limits of newly defined species.

Baird et al. (2008, 2009) found consistent genetic evidence in mtDNA, as well as autosomal and Y chromosome markers for multiple distinct lineages of what is currently referred to as the single species, *R. tumida* (Genoways and Baker1996). Their genetic data showed a distinct lineage from the Pacific versant of Mexico and Central America, a second lineage from the Atlantic versant of Mexico, and a third lineage from the Atlantic versant of Central America. None of these DNA sequence-based datasets (mtDNA – Baird et al. 2008; nuclear – Baird et al. 2009) indicated evidence for genetic introgression between any of the three lineages of *R. tumida*. Therefore, the authors hypothesized that the three genetic lineages may each represent distinct species as defined by the Genetic Species Concept.

The genetic divergence between the different lineages of *R. tumida* is significant. With cytochrome-*b* (*Cyt*b), Baird et al. (2008) reported a genetic difference

of about 10% (K2P) between the Pacific and each Atlantic lineage. The two Atlantic lineages differed from one another by about 2.5% at that locus. With ZFY (Y chromosome locus), Baird et al. (2009) found that the Atlantic Mexican *R. tumida* and Pacific *R. tumida* (along with *R. aeneus*) shared a single haplotype, whereas the Central American *R. tumida* was distinct. With MPI (autosomal locus), Baird et al. (2009) showed, again, that the Pacific *R. tumida* group was quite distinct from the other two, but did not form a statistically supported monophyletic group at this locus. The degree of differentiation found in between the Pacific and Atlantic lineage is about the level consistent with between-species comparisons in many mammalian groups (Bradley and Baker 2000). The difference between the two Atlantic lineages is lower in *Cyt*b, more typical of species within *Platyrrhinus* (Velazco et al. 2008). However, each is evidently an independently-evolving monophyletic group based on total evidence from mtDNA and nuclear loci.

Examination of the availability of species-level names from populations of the genetically distinct Central American *R. tumida* taxon, a previously described subspecies of *R. tumida*, *R. tumida major*, is now considered to be a synonym of *R. parvula* based on morphology (LaVal 1973; Simmons 2005). Another previously described subspecies of *R. tumida*, *R. tumida riparia*, is now a synonym of *R. io*, as is the former species *R. bombyx* (Genoways and Baker 1996; Simmons 2005). Therefore, based on our search of the literature, no species-level available names are applicable to the new putative species.

The type specimen of *R. tumida* is from Mirador in Veracruz, Mexico (Allen 1866). This locality is near the Atlantic coast of Mexico, in the closest geographic proximity to the genetic "Atlantic Mexican *R. tumida* lineage" of Baird et al. (2008, 2009).

The taxonomy of the *Rhogeessa tumida* complex has been exceptionally difficult to resolve. This paper is intended to examine all available data with respect to members of that complex in order to determine the geographic structure of species boundaries. We review available genetic data (nuclear and mtDNA sequences and karyotypes) in the context of the Genetic Species Concept (Baker and Bradley 2006). Additionally, we examine in more detail the presence or absence of morphological differentiation between lineages currently classified as *R. tumida*. Specifically, we examine the morphometric variation of the three genetic lineages of *R. tumida* presented in Baird et al. (2008, 2009). We also review the taxonomy of *R. tumida* and formally describe two of the distinct genetic lineages of what were previously classified as *R. tumida* as distinct species.

MATERIALS AND METHODS

Morphometric data.—Twenty specimens referred to as the "Pacific *R. tumida* lineage" and "Central American *R. tumida* lineage" by Baird et al. (2008, 2009) were examined using linear morphometric analyses (n = 17 and n = 3, respectively). In addition, seventeen specimens of six species of *Rhogessa* were used as comparative material (Appendix I). All the specimens examined had been studied genetically (Fig. 1; also see Baird et al. 2008, 2009), with the exception of additional material of *R. genowaysi* and *R. parvula*. For our purposes, it was critical to examine specimens that had been identified genetically due to the known morphological similarity of other *Rhogeessa* species. This reduced our sample size, but it removed the possibility of any identification errors. Four external measurements were recorded of skin tags or field notes information and included: overall total length (TL); length of tail (LT); length of hind foot (LHF); and length of ear (LE). Six cranial and mandibular measurements were taken to the nearest 0.01 mm with a digital caliper. Cranial and mandibular measurements were selected following LaVal (1973) with additions as noted below: greatest length of the skull (GLS—including incisors); condylobasal length (CBL); mastoid width (MW); depth of the braincase (DB); zygomatic width (ZW); postorbital width (POW); width across first upper canines (C1-C1); width across first upper incisors (I1-I1); width across second upper molars (M2-M2); maxillary length (MAXL); maxillary toothrow (MAXT); palatal length (PL); mandible length (ML—including incisors); coronoid height (CH); mandibular toothrow (MAND); and width across first lower canines (c1-c1).

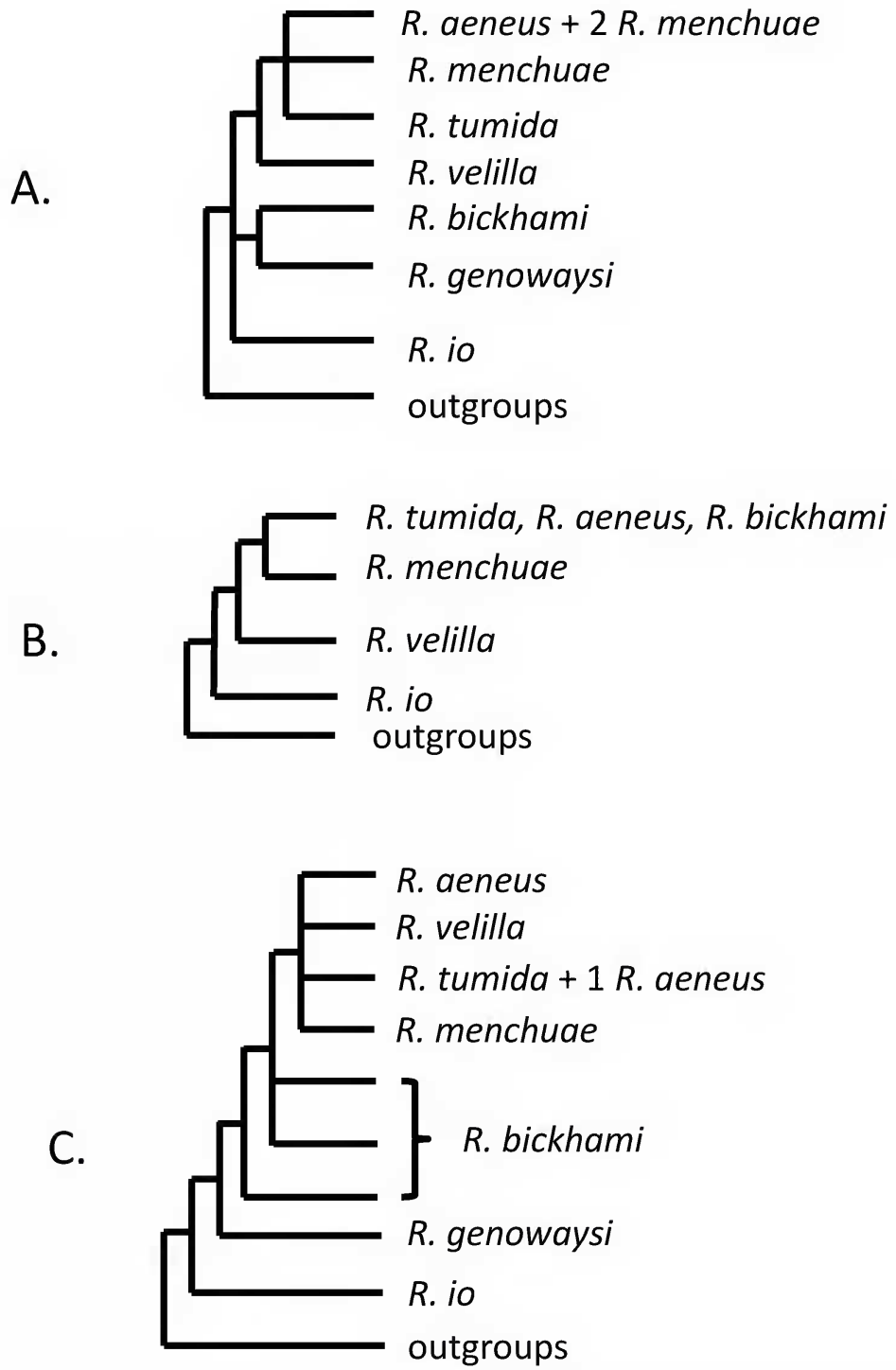

Figure 1. Representative phylogenies from previous genetic studies. A) phylogeny based on mtDNA cytochrome-*b* sequences; B) phylogeny based on Y-chromosomal ZFY sequences (note that no male *R. genowaysi* was included in this phylogeny); C) phylogeny based on nuclear MPI sequences. In these figures, all major clades have been collapsed into a single lineage. For more detailed phylogenies, see Baird et al. (2008, 2009).

Descriptive statistics (including mean, standard deviation, and range) of external, cranial, and mandibular measurements were calculated for all species.

Multivariate analysis.—Only cranial and mandibular measurements were used in multivariate analyses to eliminate measurement error (Blackwell et al. 2006). A MANOVA test was used to assess morphometric differences in all 16 measurements among species. Alpha levels were adjusted using a Bonferroni correction for multiple tests. Additionally, we performed a principal component analysis (PCA) on the covariance matrix of log-transformed cranial and mandibular measurements to establish the variation in the sample examined. Skull size variation in the sample was summarized by the first two axes of PCA (hereafter referred as PC). All analyses were conducted using MATLAB (version 6.5, The Mathworks) and SPSS (version 16.0.1, SPSS Inc.).

Phylogenetic relationships.—We examined the phylogenies of *Rhogeessa* presented in Baird et al. (2008, 2009). We examined the sequences used to generate those phylogenies to find fixed nucleotide differences between taxa. We also reviewed all available karyotypic (Bickham and Baker 1977; Baker et al. 1985), allozyme (Baker et al. 1985), and previous morphological analyses in the genus (LaVal 1973). The purpose of gathering all available phylogenetic data is to examine evidence for monophyly of putative new species of *Rhogeessa*.

RESULTS

Results of a MANOVA test showed that two of 16 cranial and mandibular measurements (zygomatic width and postorbital width) were significantly different among species of *Rhogeessa* ($P \leq 0.05$; Table 1). Mean values of zygomatic width were higher in *R. tumida sensu stricto*, *R. velilla*, and *R. io*; and lower in *R. aeneus* with respect to genetically identified phylogroups of the respective "Pacific and Central American *R. tumida* lineages" (Table 1).

A PCA of 16 cranial and mandibular measurements performed for 37 specimens found that the first two principal components accounted for 59.1% of the total variation in the sample (33.7% and 25.4%, respectively; Table 2). PC1 was highly and positively correlated with all measurements and was therefore interpreted as an overall size variation vector. Along PC1 and PC2 the variables that accounted for most of the variation were the greatest length of skull and depth of the braincase, respectively (Table 2).

Morphometric size variation among all species of *Rhogeessa* was continuously distributed along PC1 with an overlapping area in the morphological space (Fig. 2). Specimens of the undescribed "Pacific and Central American *R. tumida* lineages" were mainly overlapping with respect to the rest of the species along the first two principal components. Individuals of *R. velilla* and *R. io* were partially separated with respect to the remainder of the species primarily along the PC2.

All DNA sequence data previously published on *Rhogeessa* (Baird et al. 2008, 2009) indicate that all members of the *R. tumida* complex, including the two putative new species are reciprocally monophyletic entities (Fig. 1; Table 3). There is one instance of possible ancient hybridization between *R. tumida* and *R. aeneus* (Baird et al. 2009), but the phylogenetic patterns may also be a result of incomplete lineage sorting at the loci examined. Cytochrome-*b* sequences were examined for unique, fixed differences between species (Appendix II). These data show that each species has unique, diagnostic variation in this gene.

Morphological analyses presented in this paper showed that the morphometric independence among species of *Rhogeessa* is ambiguous and intricate. Morphometric overlap in cranial and mandibular variables complicated characterizing the two genetically distinct lineages within the *R. tumida* complex (Tables 1 and 4). However, based on previous DNA sequence data and karyotypes (Baird et al. 2008, 2009; Fig. 1) that document a lack of evidence for genetic exchange between lineages of "*R. tumida*," we conclude that the proper action to best describe the biodiversity in the *Rhogeessa tumida* complex is to recognize these genetic entities as species.

Table 1. Descriptive statistics for 16 cranial and mandibular morphological measurements. Mean and standard deviation (1st row) and range (2nd row) of all morphological measurements for eight species of Rhogeessa *including the two new genetic lineages reported by Baird et al. (2008 and 2009). Acronyms for the variables are explained in the Materials and Methods. All measurements are in millimeters. Sample size is indicated under the species name.*

Variable	Pacific *R. tumida* lineage (n = 17)	Central America *R. tumida* lineage (n = 3)	*R. tumida* (n = 1)	*R. genowaysi* (n = 5)	*R. aeneus* (n = 5)	*R. parvula* (n = 2)	*R. velilla* (n = 3)	*R. io* (n = 1)
GLS	12.05 ± 0.45	12.12 ± 0.22	11.79	12.61 ± 0.46	12.03 ± 0.17	11.90 ± 0.18)	12.16 ± 0.23	12.6
	(11.22–12.99)	(11.98–12.37)		(12.03–13.16)	(11.78–12.20)	(11.77–12.03)	(12.02–12.43)	
CBL	11.35 ± 0.58	11.56 ± 0.35	10.8	12.03 ± 0.42	11.43 ± 0.23	11.45 ± 0.36	11.39 ± 0.15	11.85
	(10.18–12.47)	(11.30–11.56)		(11.50–12.50)	(11.14–11.73)	(11.20–11.71)	(11.29–11.56)	
MW	6.74 ± 0.27	6.96 ± 0.22	6.43	6.97 ± 0.32	6.65 ± 0.09	6.77 ± 0.08	6.80 ± 0.08	7.04
	(6.22–7.14)	(6.77–7.20)		(6.56–7.25)	(6.55–6.79)	(6.71–6.83)	(6.71–6.87)	
DB	7.55 ± 0.56	7.36 ± 0.92	6.33	8.10 ± 1.07	7.68 ± 0.14	8.04 ± 0.21	6.44 ± 0.05	6.9
	(6.68–8.67)	(6.30–7.93)		(6.25–8.97)	(7.57–7.91)	(7.89–8.18)	(6.38–6.48)	
ZW	6.40 ± 0.24	6.32 ± 0.15	8.22	6.68 ± 0.27	6.17 ± 0.07	6.31 ± 0.22	7.92 ± 0.14	8.54
	(5.98–6.93)	(6.20–6.48)		(6.23–6.91)	(6.08–6.25)	(6.15–6.46)	(7.76–8.01)	
POW	3.26 ± 0.13	3.43 ± 0.09	3.32	3.34 ± 0.07	3.06 ± 0.09	3.16 ± 0.06	3.47 ± 0.03	3.17
	(3.02–3.59)	(3.33–3.50)		(3.26–3.41)	(2.93–3.15)	(3.11–3.20)	(3.45–3.50)	
C1-C1	3.64 ± 0.17	3.79 ± 0.22	3.58	3.76 ± 0.28	3.63 ± 0.06	3.67 ± 0.05	3.57 ± 0.12	3.73
	(3.41–3.95)	(3.55–3.98)		(3.43–4.02)	(3.58–3.73)	(3.64–3.71)	(3.49–3.71)	
I1-I1	2.43 ± 0.22	2.24 ± 0.21	2.29	2.32 ± 0.18	2.33 ± 0.23	2.43 ± 0.03	2.51 ± 0.01	2.57
	(2.11–2.89)	(2.10–2.48)		(2.08–2.53)	(2.10–2.67)	(2.41–2.45)	(2.50–2.52)	

Table 1 (cont.).

Variable	Pacific *R. tumida* lineage (n = 17)	Central America *R. tumida* lineage (n = 3)	*R. tumida* (n = 1)	*R. genowaysi* (n = 5)	*R. aeneus* (n = 5)	*R. parvula* (n = 2)	*R. velilla* (n = 3)	*R. io* (n = 1)
M2-M2	5.31 ± 0.21 (4.90–5.76)	5.44 ± 0.27 (5.13–5.60)	5.08	5.69 ± 0.19 (5.41–5.90)	5.25 ± 0.15 (5.08–5.47)	5.20 ± 0.02 (5.18–5.21)	5.30 ± 0.05 (5.25–5.35)	5.57
MAXL	4.18 ± 0.55 (3.23–4.83)	3.75 ± 0.69 (3.31–4.55)	4.97	3.89 ± 0.49 (3.36–4.68)	4.46 ± 0.05 (4.40–4.54)	3.91 ± 0.63 (3.46–4.35)	4.55 ± 0.02 (4.53–4.57)	4.7
MAXT	4.72 ± 0.32 (4.13–5.15)	4.38 ± 0.31 (4.03–4.58)	4.56	4.85 ± 0.26 (4.56–5.23)	4.88 ± 0.08 (4.76–4.96)	4.59 ± 0.09 (4.52–4.65)	4.12 ± 0.06 (4.07–4.18)	4.25
PL	4.58 ± 0.25 (4.12–5.01)	4.38 ± 0.24 (4.10–4.53)	4.67	5.26 ± 0.69 (4.45–6.34)	4.65 ± 0.14 (4.44–4.84)	5.08 ± 0.27 (4.89–5.27)	4.76 ± 0.14 (4.63–4.90)	4.9
ML	8.40 ± 0.43 (7.75–9.42)	8.56 ± 0.22 (8.37–8.80)	7	8.71 ± 0.27 (8.34–8.91)	8.39 ± 0.10 (8.25–8.49)	8.46 ± 0.06 (8.42–8.51)	8.00 ± 0.18 (7.79–8.13)	8.62
CH	3.48 ± 0.26 (3.02–3.88)	3.59 ± 0.47 (3.24–4.12)	3.03	3.80 ± 0.33 (3.43–4.15)	3.54 ± 0.07 (3.48–3.66)	3.50 ± 0.25 (3.32–3.68)	3.21 ± 0.09 (3.13–3.30)	3.59
MAND	5.05 ± 0.29 (4.68–5.57)	5.01 ± 0.11 (4.90–5.11)	4.91	5.23 ± 0.28 (4.86–5.55)	5.08 ± 0.14 (4.91–5.22)	5.01 ± 0.30 (4.80–5.23)	5.02 ± 0.30 (4.68–5.26)	5.13
c1-c1	2.28 ± 0.15 (1.89–2.49)	2.40 ± 0.13 (2.28–2.53)	2.13	2.33 ± 0.28 (2.04–2.71)	2.36 ± 0.12 (2.22–2.53)	2.31 ± 0.02 (2.29–2.32)	2.33 ± 0.08 (2.27–2.42)	2.17

Table 2. Percentage of variance explained and factor loadings for the first two principal components (PCs) of the analysis of 16 cranial and mandibular measurements. Variables were log10-transformed and a covariance matrix was used for the analysis. Acronyms for the variables are explained in the Materials and Methods.

Variable	PC 1	PC 2
GLS	0.81	0.24
CBL	0.75	0.32
MW	0.69	0.22
DB	0.39	0.76
ZW	0.32	-0.57
POW	0.01	-0.06
C1-C1	0.70	0.32
I1-I1	0.75	-0.12
M2-M2	0.60	0.29
MAXL	0.72	-0.63
MAXT	0.63	0.22
PL	0.54	0.31
ML	0.55	0.56
CH	0.45	0.59
MAND	0.53	0.28
c1-c1	0.49	0.31
% Variance	33.7	25.4

Table 3. Presence operational criteria that justify species status in the Rhogeessa tumida *complex. SSRM = Statistically Supported Reciprocal Monophyly.*

Species	SSRM: *Cyt*b	SSRM: Y Chromosome	SSRM: MPI Locus	Karyotypically distinct	Morphologically distinct
R. tumida vs. *R. bickhami*	Yes	No	Yes	No	No
R. tumida vs. *R. menchuae*	Yes	Yes	Yes	No	No
R. tumida vs. *R. genowaysi*	Yes	Unknown	Yes	Yes	Yes
R. tumida vs. *R. aeneus*	Yes	No	No	Yes	Unknown
R. tumida vs. *R. io*	Yes	Yes	Yes	Yes	Unknown
R. tumida vs. *R. velilla*	Yes	Yes	Yes	Yes	Unknown
R. bickhami vs. R. menchuae	Yes	Yes	Yes	No	No
R. bickhami vs. *R. genowaysi*	Yes	Unknown	Yes	Yes	Yes
R. bickhami vs. *R. aeneus*	Yes	No	Yes	Yes	Unknown
R. bickhami vs. *R. io*	Yes	Yes	Yes	Yes	Unknown
R. bickhami vs. *R. velilla*	Yes	Yes	Yes	Yes	Unknown
R. menchuae vs. *R. genowaysi*	Yes	Unknown	Yes	Yes	Yes
R. menchuae vs. *R. aeneus*	No	Yes	Yes	Yes	Unknown
R. menchuae vs. *R. io*	Yes	Yes	Yes	Yes	Unknown
R. menchuae vs. *R. velilla*	Yes	Yes	Yes	Yes	Unknown
R. genowaysi vs. *R. aeneus*	Yes	Unknown	Yes	Yes	Yes
R. genowaysi vs. *R. io*	Yes	Unknown	Yes	Yes	Yes
R. genowaysi vs. *R. velilla*	Yes	Unknown	Yes	No	Yes
R. aeneus vs. *R. io*	Yes	Yes	Yes	Yes	Unknown
R. aeneus vs. *R. velilla*	Yes	Yes	Yes	Yes	Unknown
R. io vs. *R. velilla*	Yes	Yes	Yes	Yes	Unknown

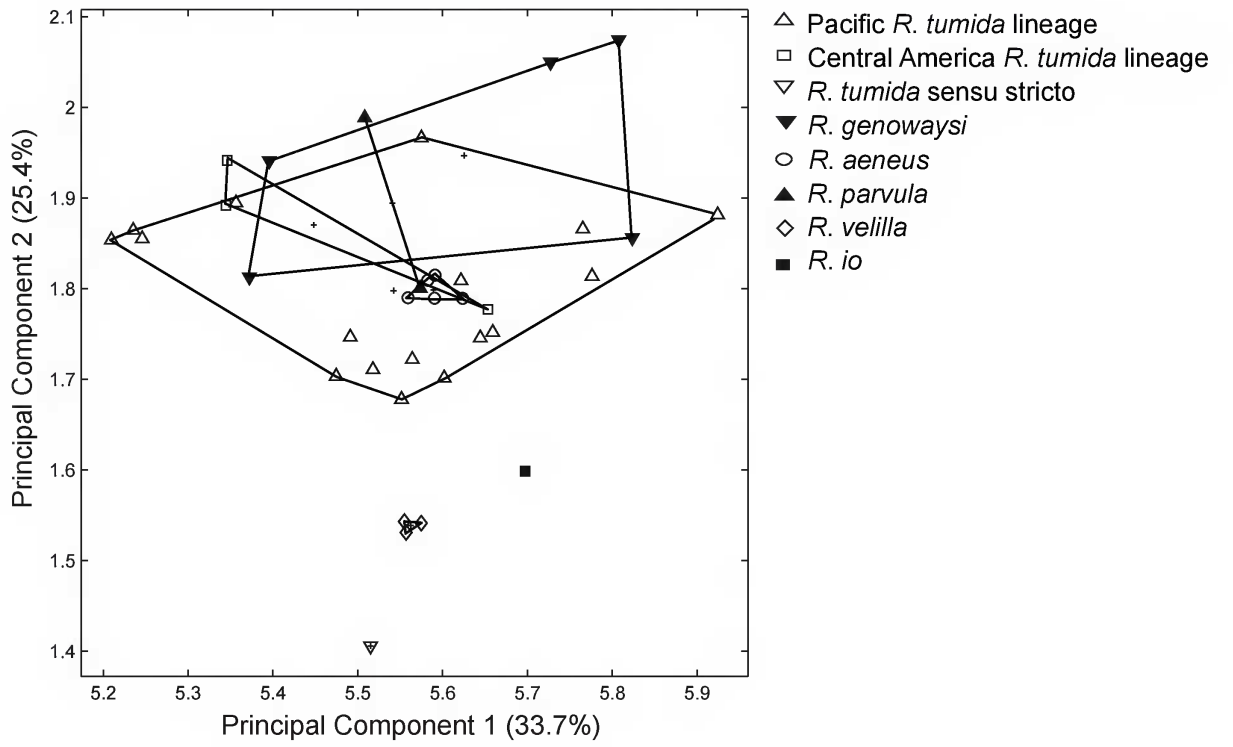

Figure 2. Results of a principal component analysis (PCA) showing the spatial position of individuals of *Rhogeessa* based on 16 cranial and mandibular measurements. Skull size variation is summarized by the first principal component (PC1), showing overlap among taxa along this axis.

Table 4. Descriptive statistics for four external morphological measurements. Mean and standard deviation (1st row) and range (2nd row) of all morphological measurements for eight species of Rhogeessa *including the two new genetic lineages reported by Baird et al. (2008, 2009). Acronyms for the variables are explained in the Materials and Methods. All measurements are in millimeters. Sample size is indicated under the species name.*

Variable	Pacific *R. tumida* lineage (n = 16)	Central America *R. tumida* lineage (n = 3)	*R. tumida* (n = 1)	*R. genowaysi* (n = 1)	*R. aeneus* (n = 4)	*R. parvula* (n = 1)	*R. velilla* (n = 3)	*R. io* (n = 1)
TL	72.13 ± 3.16	73.00 ± 3.61	78	82	74.25 ± 2.22	70	67 ± 1.73	72
	(66–78)	(69–76)			(72–77)		(65–68)	
LT	29.63 ± 3.28	31.00 ± 1.73	30	30	33.25 ± 1.71	30	26 ± 3.46	30
	(22–36)	(29–32)			(31–35)		(22–28)	
LHF	6.19 ± 0.66	5.67 ± 1.15	6	7	6.00 ± 0	4	7.67 ± 2.08	5
	(5–7)	(5–7)					(6–10)	
LE	12.44 ± 0.81	10.67±1.15	12	11	13.00 ±0.82	12	13.00 ± 2.65	11
	(11–14)	(10–12)			(12–14)		(11–16)	

DESCRIPTIONS

Family Vespertilionidae Gray 1821
Genus *Rhogeessa* H. Allen 1866
***Rhogeessa bickhami*, new species**

Holotype.—Adult female, TTU-M36161 deposited at the Natural Science Research Laboratory, Museum of Texas Tech University (Fig. 3). Holotype preserved as skin, with skull extracted in good condition. Specimen collected by L. W. Robbins (collector's number 10594) on 20 May 1981. Collector's measurements (in mm) recorded on skin tag: overall total length, 72; length of tail, 30; length of hind foot, 5; and length of ear, 13. Cranial and mandible measurements taken by MRMR: greatest length of the skull, 12.06; condylobasal length, 11.04; mastoid width, 6.73; depth of the braincase, 7.51; zygomatic width, 6.37; postorbital width, 3.25; width across first upper canines, 3.72; width across first upper incisors, 2.54; width across second upper molars, 5.28; maxillary length, 4.55; maxillary toothrow, 5.07; palatal length, 4.62; mandible length, 8.35; coronoid height, 3.29; mandibular toothrow, 5.19; and width across first lower canines, 2.43. Nucleotide sequence of the mitochondrial *Cyt*b gene deposited in GenBank with accession number EF222338 and the nuclear MPI gene as EU220356 and EU220357 (the holotype possessed two different alleles at the MPI locus).

Type locality.—23.6 mi N Huixtla, Chiapas, Mexico (Fig. 4). This is the exact type locality of *Rhogeesa genowaysi* (Baker 1984) and the two species are sympatric, even being taken in the same mist net at the same time.

Type series (16).—Sixteen individuals (12 females and 4 males) are included in the type series: TTU-M36164 (adult female, skin and a skull preparation in good condition), specimen collected by R. L. Robbins (collector's number 1224) on 21 May 1981 at 23.6 mi N Huixtla, Chiapas, Mexico; TTU-M60985 (adult female, skin and skull preparation in good condition), specimen collected by J. G. Owen (collector's number 586) on 30 October 1990 at Hacienda Escuintla, Zacatecoluca, Department of La Paz, El Salvador; TTU-M60986 (adult female, skin and skull preparation in good condition) and TTU-M60987 (adult female, skin and skull preparation in good condition), specimens collected by J. G. Owen (collector's numbers 480 and 481, respectively) on 20 July 1990 near El Guaje, Department of San Salvador, El Salvador; TTU-M83681 (adult female; skin, skull and skeleton in good condition), specimen preparation by R. Van Den Bussche, collector's number 1833; TTU-M83682 (adult male; skin, skull and skeleton in good condition), specimen preparation by S. R. Hoofer, collector's number 799; TTU-M83705 (adult female; skin, skull and skeleton in good condition), specimen preparation by B. R. Amman, collector's number 85; TTU-M83713 (adult female; skin, skull and skeleton in good condition), specimen preparation by R. D. Bradley, collector's number 1419; TTU-M83927 (adult female; skin, skull and skeleton in good condition) specimen preparation by R. Van Den Bussche, collector's number 1865 -- previous five listed specimens were collected at 3 km N, 12.5 km SW San Lorenzo, Department of Valle, Honduras; TTU-M84027 (adult male; skin, skull, and a skeleton preparation in good condition) and TTU-M84030 (adult female; skin, skull, and skeleton preparation in good condition), specimens preparation by R. D. Bradley and B. R. Amman (collector's numbers 1434 and 126, respectively) on 11 July 2001 at Comayagua (Senasa), Department of Comayagua, Honduras; TCWC-47833 (adult female; preserved in alcohol, skull removed in good condition), collected by T. J. McCarthy (collector's number 6737) on 4 February 1983 at Finca La Pacifica, Guanacaste, Costa Rica; TCWC-49791 (adult male; preserved in alcohol, skull removed in good condition), collected by R. D. Bradley, J. Ensink, and T. Lee (collector's number 231); TCWC-49793 (adult male; preserved in alcohol, skull removed in good condition), collected by R. D. Bradley, J. Ensink, and T. Lee (collector's number 233); TCWC-49797 (adult female; preserved in alcohol, skull removed in good condition), collected by R. D. Bradley, J. Ensink, and T. Lee (collector's number 339); and TCWC-49799 (adult female; preserved in alcohol, skull removed in good condition), collected by R. D. Bradley, J. Ensink, and T. Lee (collector's number 384) on June 1984 -- previous four specimens collected on June 1984 at 2.6 mi W, 10.8 mi S Jícaro Galan, Department of Valle, Honduras. The nucleotide sequences of the mitochondrial gene *Cyt*b of all type specimens have been deposited in GenBank (accession numbers are in Appendix I).

Figure 3. Dorsal, ventral, and lateral view of the skull and lower jaw of the holotype of (A) *Rhogeessa bickhami* (TTU-M36161) and (B) *R. menchuae* (TTU-M61230).

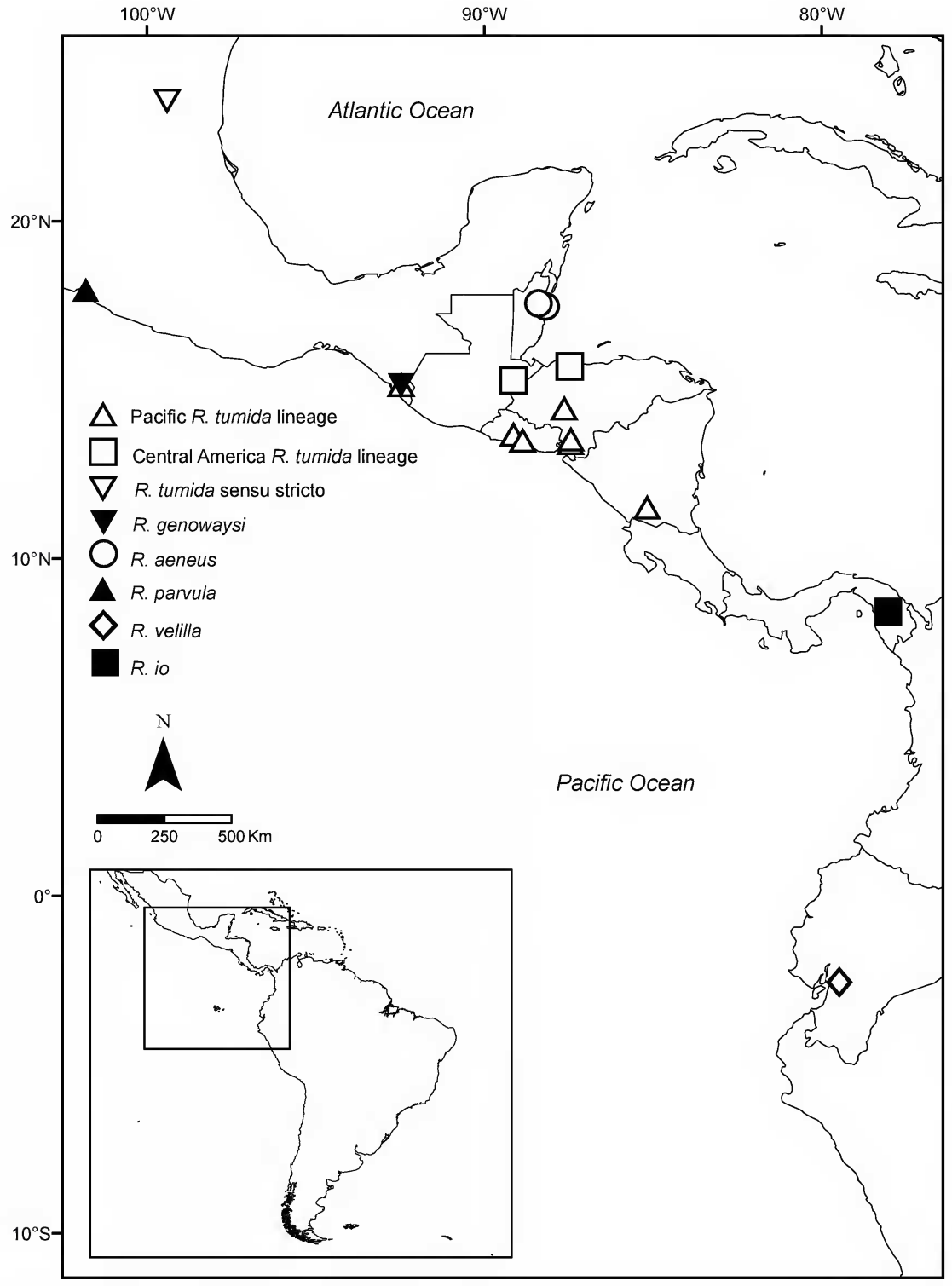

Figure 4. Collecting localities of specimens examined for morphological analysis of species of *Rhogeessa* included in this study.

Distribution.—From previous molecular studies (Baird et al. 2008, 2009), *R. bickhami* was referred to as the "Pacific *R. tumida* lineage." It is known to inhabit the Pacific versant of Middle America from Chiapas, Mexico, to Guanacaste in Costa Rica (and perhaps into Panama; Fig. 4). The only record of *R. bickhami* from the Atlantic side of the Central American mountain ranges, is from the western side of the Motagua Valley in Guatemala and the Comayagua Valley in Honduras (see Figure 1 in Baird et al. 2008 for detailed locality information).

Etymology.—This species is named for John W. Bickham, in recognition of his many years of contributions to the study of *Rhogeessa* (and other mammalian species), and his role in the description of the monobrachial model of chromosomal speciation (Bickham and Baker 1977; Baker and Bickham 1986). He has been a mentor to many aspiring mammalogists, including a life-long mentor and role model to ABB. We recommend "Bickham's little yellow bat" as the English common name.

Diagnosis.—*Rhogeessa bickhami* has a karyotype of 2n = 34 (Bickham and Baker 1977). This species comprises the "Pacific *R. tumida* lineage" outlined in Baird et al. (2008; based on cytochrome-*b* sequences) and Baird et al. (2009; based on two nuclear loci). Morphologically, *R. bickhami* is a medium-sized species of *Rhogeessa* (overall total length 66–78 mm; Table 4). The tips of dorsal fur are intense dark-colored brown or black, with bases usually buffy gray to buffy yellow (Ridgway 1912; LaVal 1973). The ventral fur is light brown. No furry fringe is present along the uropatagium. The ears are short (length of ear 11–14 mm; Table 4) and dark-colored. Overall skull size is small (greatest length of skull 11.22–12.99 mm; Table 1). The rostrum is narrower than the globular braincase. The forehead slope is slight and helmet is present above the occiput. The posterior parietal sinus is absent. The postorbital process is greatly reduced and the sagittal crest of sagittal suture of posterior part of braincase is elevated. Basisphenoid pits are absent. The cingula of canines are convex and laterally exhibit two well-developed lobes. The body of mandible is straight. The coronoid process is large, triangular, and vertical. The condyloid process is rounded and the angular process is short and slim. The dental formula is i 1/3, c 1/1, p 1/2, m 3/3, total 30.

Rhogeessa bickhami is similar in body and skull size respect to *R. menchuae* (Table 1) and both are smaller than *R. genowaysi*, which is the largest size species in the subgenus. In skull shape, all species are similar and difficult to distinguish without genetic data. In the ventral view, one of the differences found among the species is the size and proportion of the auditory bullae, and the shape and disposition of the molars. *Rhogeessa genowaysi* has a short hypocone in M2, and M3 is 3/4 of the M2 in length and 1/3 in width. *Rhogeessa bickhami* and *R. menchuae* have similar patterns of dental morphology with wider hypocones and M3 almost the same length as M2.

Habitat and ecology.—This species inhabits the semi-arid Pacific coastal plains of northern Central America (Chiapas to Costa Rica, possibly to Panama), but extending also to the Atlantic slope through the semi-arid intermontane valleys of Guatemala and Honduras. Much of the area is dominated by moist tropical deciduous forest, and dry tropical forest. There are two well marked seasons, one rainy from May to October, with intense episodes of rainfall. The other well marked season is primarily dry and extents from November to April. Typical trees found in the inter-mountain valleys in Guatemala include *Bursera*, *Ceiba*, *Acacia*, *Spondias*, and *Cordia*, among others. The forest frequently does not exceed 15 meters tall in height. More xerophitic conditions are found at the intermountain valleys, including the Motagua Valley in Guatemala, the driest locality in Central America (ca. 400 mm of annual rainfall), where columnar cacti are a characteristic of the local ecology. Despite the generally dry conditions that may be associated with this species, *R. bickhami* has been captured close to and within gallery forest along the rivers, as well as in the plant assemblages associated with homesteads of small farms with introduced and natural trees and domestic animals, such as cows, chickens, etc., and agricultural activities.

***Rhogeessa menchuae*, new species**

Holotype.—Adult male, TTU-M61230 deposited at the Natural Science Research Laboratory, Museum of Texas Tech University (Fig. 3). Holotype preserved in alcohol, with skull extracted and zygomatic arches broken. Specimen collected by R. D. Bradley (collector's number 612) on 06 August 1991. Collector's

measurements (in mm) recorded on skin tag are: total length, 69; length of tail, 29; length of hind foot, 5; and length of ear, 10. Cranial and mandible measurements taken by MRMR are: greatest length of the skull, 11.98; condylobasal length, 11.96; mastoid weight, 6.77; depth of the braincase, 7.93; zygomatic width, 6.28; postorbital weight, 3.33; weight across first upper canines, 3.55; weight across first upper incisors, 2.13; weight across second upper molars, 5.13; maxillary length, 3.31; maxillary toothrow, 4.54; palatal length, 4.51; mandible length, 8.5; coronoid height, 3.24; mandibular toothrow, 5.03; and weight across first lower canines, 2.28. Nucleotide sequence data deposited in GenBank with the following accession numbers: mitochondrial *Cyt*b EF222378, ZFY EU185117, and MPI EU220348.

Type locality.— Lancetilla, Department of Atlántida, Honduras (Fig. 4).

Type series (2).—Type series includes: TTU-M61229 (adult female, preserved in alcohol and skull extracted with braincase broken), specimen collected by R. D. Bradley (collector's number 597) on 5 August 1991 at Lancetilla, Department of Atlántida, Honduras; and USAC-4396 (adult female, preserved as skin and skull with zygomatic arch broken), specimen collected by Sergio Guillermo Pérez Consuegra (collector's number 1305) on 26 July 2006 at Rio Vega Grande, Los Amates, Department of Izabal, Guatemala (299 m above sea level). The nucleotide sequences of the mitochondrial *Cyt*b gene of type specimens were deposited in GenBank (Appendix I).

Distribution.— From previous molecular studies (Baird et al. 2008, 2009), *R. menchuae* was referred to as the "Central America *R. tumida* lineage." *Rhogeessa menchuae* is known to occur from the northern limit on the Caribbean coast of Guatemala (near the city of Puerto Barrios) to the southernmost locality documented from genetic data on the Atlantic coast of Honduras, near the Guatemalan border. The distribution of *R. menchuae* likely extends further south into Central America, perhaps as far south as Nicaragua.

Etymology.—This species is named to honor Rigoberta Menchú (along with the rest of the Menchú family) for her decades of work establishing a better understanding of the Mayan culture in Guatemala. Her important work has earned her a Nobel Peace Prize. She always underscored, among other traits, the respect of nature by the native peoples of this area. We propose "Menchu's little yellow bat" as the English common name.

Diagnosis.—*Rhogeessa menchuae* has a karyotype of 2n = 34 (Bickham and Baker 1977) and is referred in Baird et al. (2008; based on *Cyt*b) and Baird et al. (2009; based on nuclear genes) as the "Central American *R. tumida*" lineage. *Rhogeessa menchuae* is a medium-size species of *Rhogeessa* (overall total length 69–76 mm; Table 4), and is similar in size and form to *R. bickhami* and *R. tumida* (Fig. 3; Tables 1 and 4). Externally, the tip of dorsal fur is bicolored from dark to light brown with bases buffy yellow. The ventral fur varies from light to dark brown. Fur is present at the base of the uropatagium. The ears are short (length of ear 10–12 mm; Table 4) and dark-colored. The skull size is small (greatest length of skull 11.98–12.37 mm). The rostrum is flattened in orbital region, and narrower than globular braincase. The forehead slope is slight. The helmet is present above occiput. The postorbital width is narrow in relation to the skull size. The sagittal crest of sagittal suture of posterior part of braincase elevated. The basisphenoid pits are absent. The infraorbital foramens are projected frontally. The canines are large, and incisors are procumbent. The cingulum of C1 is well developed, with two accessory cuspids. A gap is present between M1-M2 and M2-M3. The upper and lower central incisors are convergent. There is a narrow distance between the C1-C1. The body of mandible is straight. The coronoid process is large, triangular, and vertical. The angular process is short. The first and second lower incisors, i1 and i2, are tricuspid with lateral posterior cusps smaller, and i3 is unicuspid. The dental formula is i 1/3, c 1/1, p 1/2, m 3/3, total 30.

In size, *R. menchuae* overlaps in external and skull measurements with *R. bickhami* (see Table 1 and 4), and it is larger than *R. parvula* and *R. aeneus*. Relative to the morphology of *R. bickhami*, *R. menchuae* has a lighter pelage and ears, a more elevated angular projection, a shorter rostrum, the upper incisors larger and thinner, and M3 smaller. Its dentition is similar to *R. bickhami* and *R. tumida*, but is less robust than *R. genowaysi*. The cingulum of C1 is more developed in *R. genowaysi*, *R. aeneus*, and *R. io*. The size of

i3 is smaller in *R. aeneus*, *R. parvula*, and *R. velilla* compared with *R. bickhami*, *R. genowaysi*, *R. io*, *R. menchuae*, and *R. tumida*.

Habitat and ecology.—This species inhabits the humid Atlantic coastal region of northern Central America, certainly Guatemala and Honduras, but possibly also southern Belize and northern Nicaragua. The area is characterized by humid conditions, high annual rainfall rates and tall tropical rainforest, with trees such as *Ficus*, *Callophyllum*, *Pouteria*, *Vochisia*, and many others. The most common palm is *Orbignya*, and many kinds of epiphytes are found over the tall trees that may reach near 30 meters. There is only a short dry season, and a long rainy season that extends from May to January. In Izabal, Guatemala, where this species has been collected, the typical annual rainfall is around 4,000 mm. The area is largely cultivated and many towns and human settlements are present in the area especially near the gulf coast. Although the distribution of this species is geographically adjacent to that of *R. bickhami* in Guatemala, it has not been collected in sympatry, a situation that may be similar in every dry valley that turns into humid conditions in surrounding areas, mainly in Honduras.

DISCUSSION

The addition of the two species described above brings the total number of species in the *Rhogeessa tumida* complex to eight. The six previously described species include: 1) *R. tumida* (Allen 1866; type locality: Mirador, Veracruz, Mexico; distribution: the Atlantic versant of Mexico from Tamaulipas to the Isthmus of Tehuantepec); 2) *R. genowaysi* (Baker 1984; type locality: 23.6 miles northwest of Huixtla, Chiapas, Mexico; distribution: only known from type locality); 3) *R. io* (Thomas 1903; type locality: Valencia, Venezuela; distribution: southern Panama to northern South America); 4) *R. velilla* (Thomas 1903; type locality: Puná Island, Ecuador; distribution: known from mainland Ecuador and the type locality); 5) *R. aeneus* (Goodwin 1958; type locality: Chichen Itza, Yucatán, Mexico; distribution: Yucatán and Campeche, Mexico, Belize, and Petén, Guatemala); and 6) *R. hussoni* (Genoways and Baker 1996; type locality: Sipaliwini Airstrip, Nickerie District, Suriname; distribution: northeastern South America).

The *Rhogeessa tumida* complex appears to exhibit an unusual amount of species diversity in the absence of morphological differentiation. *Rhogeessa genowaysi* was the first member of the *R. tumida* complex to be elevated to species status based on genetic (specifically, karyotypic rearrangements involving at least 3 pairs of chromosomes) differences in sympatric individuals without evidence of hybrids (Baker 1984). Subsequent species also were described mainly based on karyotypic differences (Genoways and Baker 1996; Audet et al. 1993). Baird et al. (2008) and Baird et al. (2009) demonstrated that the descriptions of these species was supported by DNA sequence data from mitochondrial, nuclear and Y chromosome genetic markers, as they all form reciprocally monophyletic clades using the three different genetic markers, with only a single observed instance of ancient hybridization between *R. aeneus* and *R. tumida* (although the authors caution that the observed phylogenetic patterns could simply be due to incomplete lineage sorting at the loci examined). Despite the fact that no karyotypic differences exist between *R. tumida*, *R. bickhami*, and *R. menchuae*, no evidence of gene flow was detected between these species. In fact, in no genetic locus examined was *R. tumida* (by this definition, including *R. menchuae* and *R. bickhami*) found to be a monophyletic group (Baird et al. 2008, 2009). Therefore, we believe that the two new species described here represent well-supported genetic species under the definition given in Baker and Bradley (2006).

Because little is known about reproductive behavior in *Rhogeessa*, combined with the lack of morphological differentiation between species, it is a particularly difficult task to determine the proper taxonomy of this group. Previous allozymic and karyotypic studies were able to support the elevation of *R. genowaysi*, *R. aeneus*, *R. io*, and *R. hussoni*. With new DNA sequence data from multiple loci (Baird et al. 2008, 2009), these four species were additionally confirmed. This DNA sequence data also demonstrated the existence

of *R. velilla*, which is karyotypically identical to *R. genowaysi*, although widely separated geographically. Although *R. velilla* and *R. genowaysi* share a diploid value (2n=42) and karyotypic morphology, the two are not sister species in the mitochondrial and nuclear gene trees (Baird et al 2008, 2009). The most surprising evidence from these molecular phylogenies was in identifying three genetically distinct lineages within the karyotypically identical "*R. tumida*." All previously documented species of *Rhogeessa* were confirmed using the genetic markers summarized above. Because the two new species described here show similar patterns demonstrating independently evolving lineages, they merit recognition as distinct species.

Broader impacts.—The results from the analyses show that cryptic species within some groups of mammals might be more abundant than previously believed. Without genetic analyses, the now eight species within the *R. tumida* complex would still be considered a conspecific widely distributed single species. The description of *R. genowaysi* as specifically distinct from *R. tumida* based on karyotypic differences in sympatry without hybrids was one of the first cryptic species discovered in mammals (Baker 1984; cryptic species of mammals were reviewed in Baker and Bradley 2006). Since then, other cryptic species of mammals have been discovered but are still relatively rare, although the use of molecular genetics techniques is allowing easier identification of cryptic species (Ceballos and Ehrlich 2009). Our study indicates that this phenomenon may be even more common than previously proposed by Baker and Bradley (2006), who suggested that 2,000 unrecognized species may be present in the third edition of Mammal Species of the World (Wilson and Reeder 2005). While we do not yet fully understand the mechanisms that facilitate certain groups being more prone to speciation in the absence of morphological variation, genetic incompatibilities are likely responsible as the isolating mechanism in many instances especially in the case of *Rhogeessa.*

Studies such as the one reported here have important conservation implications. For example, *Rhogeessa genowaysi* is only known from two localities in Mexico and is currently on the 2010 IUCN endangered species list. Had this species never been described, its extinction due to habitat loss would probably have occurred, perhaps without knowledge of its existence (Baird 2010).

The conservation status of *R. bickhami* and *R. menchuae* remain to be determined and will require substantial study. A result of the eight species being so difficult to tell apart under field conditions is that genetic studies will need to be carried out to determine the species boundaries, geographical distributions, and relative abundance of all species of *Rhogeessa.* Such basic research will be the foundation needed to understand and protect those unique species. We are only beginning to understand this complex of bats.

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LITERATURE CITED

Allen, H. 1866. Notes on the Vespertilionidae of tropical South America. Proceedings of the Academy of Natural Sciences of Philadelphia 18:279-288.

Audet, D., M. D. Engstrom, and M. B. Fenton. 1993. Morphology, karyology, and echolocation calls of *Rhogeessa* (Chiroptera: Vespertilionidae) from the Yucatan Peninsula. Journal of Mammalogy 74:498-502.

Baird, A. B., D. M. Hillis, J. C. Patton, and J. W. Bickham. 2008. Evolutionary history of the genus *Rhogeessa* (Chiroptera: Vespertilionidae) as revealed by mitochondrial DNA sequences. Journal of Mammalogy 89:744-754.

Baird, A. B., D. M. Hillis, J. C. Patton, and J. W. Bickham. 2009. Speciation by monobrachial centric fusions: A test of the model using nuclear DNA sequences from the bat genus *Rhogeessa*. Molecular Phylogenetics and Evolution 50:256-267.

Baird, A. B. 2010. Genetic identification of cryptic species: An example in *Rhogeessa*. Pp. 22-23 in Molecular approaches in natural resource conservation and management (J. A. DeWoody, J. W. Bickham, C. H. Michler, K. M. Nichols, O. E. Rhodes, Jr., and K. E. Woeste, eds.). Cambridge University Press, Cambridge, Massachusetts.

Baker, R. J. 1984. Mammalian sympatric, cryptic species: A new species of *Rhogeessa* (Chiroptera: Vespertilionidae). Systematic Zoology 32:178-183.

Baker, R. J., J. W. Bickham, and M. L. Arnold. 1985. Chromosomal evolution in *Rhogeessa* (Chiroptera: Vespertilionidae): Possible speciation by centric fusions. Evolution 39:233-243.

Baker, R. J., and J. W. Bickham. 1986. Speciation by monobrachial centric fusions. Proceedings of the National Academy of Science 83:8245-8248.

Baker, R. J., and R. D. Bradley. 2006. Speciation in mammals and the genetic species concept. Journal of Mammalogy 87:643-662.

Bickham, J. W., and R. J. Baker. 1977. Implications of chromosomal variation in *Rhogeessa* (Chiroptera: Vespertilionidae). Journal of Mammalogy 58:448-453.

Blackwell, G. L., S. M. Basset, and C. R. Dickman. 2006. Measurement error associated with external measurements commonly used in small-mammal studies. Journal of Mammalogy 87:216-223.

Bradley, R. D., and R. J. Baker. 2000. A test of the Genetic Species Concept: Cytochrome-*b* sequences and mammals. Journal of Mammalogy 82:960-973.

Ceballos, G., and P. R. Ehrlich. 2009. Discoveries of new mammal species and their implications for conservation and ecosystem services. Proceedings of the National Academy of Sciences 106:3841-3846.

Genoways, H. H., and R. J. Baker. 1996. A new species of the genus *Rhogeessa*, with comments on geographic distribution and speciation in the genus. Pp. 83-87 in Contributions to Mammalogy: A Memorial Volume Honoring Dr. J. Knox Jones, Jr. (H. H. Genoways and R. J. Baker, eds.). Museum of Texas Tech University, Lubbock, Texas.

Goodwin, G. G. 1958. Bats of the genus *Rhogeessa*. American Museum Novitates 1923:1-17.

Hellborg, L., I. Gündüz, and M. Jaarola. 2005. Analysis of sex-linked sequences supports a new mammal species in Europe. Molecular Ecology 14:2025-2031.

Larsen, P. A., M. R. Marchan-Rivadeneira, and R. J. Baker. 2010. Natural hybridization generates mammalian lineage with species characteristics. PNAS 107(25):11447-11452

Lausen, C. L., I. Delisle, R. M. R. Barclay, and C. Strobeck. 2008. Beyond mtDNA: Nuclear gene flow suggests taxonomic oversplitting in the little brown bat (*Myotis lucifugus*). Canadian Journal of Zoology 86:700-713.

LaVal, R. K. 1973. Systematics of the genus *Rhogeessa* (Chiroptera: Vespertilionidae). Occasional Papers of the Museum of Natural History, The University of Kansas, Lawrence, Kansas 19:1-47.

Ridgway, R. 1912. Color standards and color nomenclature. Privately published, Washington, D.C.

Thomas, O. 1903. Two South American forms of *Rhogeessa*. Annals and Magazine of Natural History 7(11):382-383.

Velazco, P., and B. D. Patterson. 2008. Phylogenetics and biogeography of the broad-nosed bats, genus *Platyrrhinus* (Chiroptera: Phyllostomidae). Molecular Phylogenetics and Evolution 49:749-759

Wilson, D. E., and D. M. Reeder (eds). 2005. Mammal Species of the World: A Taxonomic and Geographic Reference (3rd edition). Johns Hopkins University Press, Baltimore, Maryland. 2,142 pp.

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Appendix I

Specimen, locality, tissue number, catalog number, and GenBank accession number of 37 specimens used in morphometric analysis. An asterisk (*) indicates specimens for which external measurements were not available.

Species	Museum No.	Tissue No.	Country	Locality	GenBank Accession Nos.
R. aeneus	TTU-M40003	TK 20704	BELIZE	Belize District	EF222329, EU002301, EU220302
R. aeneus	TTU-M40005	TK 20707	BELIZE	Belize District	EF222361, EU220304, EU220305
R. aeneus	TTU-M40009	TK 20710	BELIZE	Belize District	EF222395, EU220306, EU220307
R. aeneus	TTU-M40010	TK 20706	BELIZE	Belize District	EF222329, EU220302
*R. aeneus**	TTU-M40012	TK 20712	BELIZE	Belize District	EF222364, EU220308, EU220309
R. bickhami new sp.*	TCWC-47833	AK 7022	COSTA RICA	Guanacaste	EF222335
R. bickhami new sp.	TCWC-49791	AK 9585	HONDURAS	Valle	EF222326
R. bickhami new sp.	TCWC-49793	AK 9587	HONDURAS	Valle	EF222372, EU185000, EU220376, EU220377
R. bickhami new sp.	TCWC-49797	AK 9615	HONDURAS	Valle	EF222373
R. bickhami new sp.	TCWC-49799	AK 9617	HONDURAS	Valle	EF222373
R. bickhami new sp. (holotype)	TTU-M36161	TK 20594	MEXICO	Chiapas	EF222338, EU220356, EU220357
R. bickhami new sp.	TTU-M36164	TK 20596	MEXICO	Chiapas	EF222356, EU220358
R. bickhami new sp..	TTU-M60985	TK 34902	EL SALVADOR	La Paz	EF222385
R. bickhami new sp.	TTU-M60986	TK 34866	EL SALVADOR	San Salvador	EF222380, EU220359, EU220360
R. bickhami new sp.	TTU-M60987	TK 34867	EL SALVADOR	San Salvador	EF222353
R. bickhami new sp.	TTU-M83681	TK 101020	HONDURAS	Valle	EF222351, EU220365, EU220366
R. bickhami new sp.	TTU-M83682	TK 101021	HONDURAS	Valle	EF222352, EU185113, EU220367, EU220368
R. bickhami new sp..	TTU-M83705	TK 101044	HONDURAS	Valle	EF222367, EU220369
R. bickhami new sp..	TTU-M83713	TK 101052	HONDURAS	Valle	EF222368, EU220370, EU220371
R. bickhami new sp.	TTU-M83927	TK 101266	HONDURAS	Valle	EF222409, EU220372, EU220373
R. bickhami new sp.	TTU-M84027	TK 101367	HONDURAS	Comayagua	EF222383, EU185128, EU220374, EU220375
R. bickhami new sp.	TTU-M84030	TK 101370	HONDURAS	Comayagua	EF222411, EU220389

APPENDIX I (CONT.)

Species	Museum No.	Tissue No.	Country	Locality	GenBank Accession Nos.
*R. genowaysi**	TTU-M29103	TK 5390	MEXICO	Chiapas	Not in GenBank
*R. genowaysi**	TTU-M29104	TK 5310	MEXICO	Chiapas	Not in GenBank
*R. genowaysi**	TTU-M29106	TK 5312	MEXICO	Chiapas	Not in GenBank
*R. genowaysi**	TTU-M29108	TK 5314	MEXICO	Chiapas	Not in GenBank
R. genowaysi	TTU-M36171	TK 20597	MEXICO	Chiapas	EF222326, EU220390
R. io	TTU39147	TK 22536	PANAMA	Darien	EF222369, EU220345, EU220346
R. menchuae new sp.	TTU-M61229	TK 40345	HONDURAS	Atlantida	EF222377, EU220347
R. menchuae new sp. (holotype)	TTU-M61230	TK 40360	HONDURAS	Atlantida	EF222378, EU185117, EU220348
R. menchuae new sp.	USAC-4396	AK 25093	GUATEMALA	Izabal	EF222415, EU220355
*R. parvula**	TTU46788	TK 4765	MEXICO	Guerrero	EF222353
R. parvula	TTU-M37726	TK 19557	MEXICO	Jalisco	Not in GenBank
R. tumida sensu stricto	TTU44867	TK 27068	MEXICO	Tamaulipas	EF222345, EU185116, EU220328
R. velilla	TTU103254	TK 134792	ECUADOR	Guayas	EF222339, EU185121, EU220331
R. velilla	TTU103292	TK 134868	ECUADOR	Guayas	EF222366, EU220333
R. velilla	TTU103525	TK 134692	ECUADOR	Guayas	EF222341, EU185120, EU220392

Appendix II

Fixed differences in cytochrome-*b* sequences for members of the *Rhogeessa tumida* complex. This table does not include variation that is not fixed for all specimens of at least one species. Numbers along the top row indicate nucleotide position. Data was obtained from GenBank sequences originally published by Baird et al. (2008).

	33	42	47	54	60	61	66	69	78	81	96	99	102	105	108	123	126	127	145	150
R. aeneus	T	T	G	C	T	T	A	T	A/C	T	C	T	G/A	C	C/T	G	A	G/A	C/T	C
R. menchuae	T	T	G	C	T	T	A	T	C	T	C	T	G/A	C	C/T	A	A	G	T	C
R. tumida	T	T	G	C	C	T	A	C	C	T	C	T	G/A	C	C	G	A	G	T	T/C
R. velilla	T	T	A	T	T	C/T	A	C	C	C	T	C	G	C	C	A	T	T	C	T
R. bickhami	T	T	G	C	C/T	C	C	T	C	C	C	C	A	C	C	A	A	T	C/T	C
R. genowaysi	C	C	G	C	T	C	C	T	C	T	C	T	G	C	C	A	A	T	C	T
R. io	C/T	T/C	A/G	C	T	T	C	T	T	T	T	C	G	T	T	C	A	T	T	C

	153	156	159	162	165	174	177	180	195	198	204	207	216	222	228	234	235	243	249	252
R. aeneus	A/G	C	A	C	T	T	C	C	T	C	C	T	C	C	A/G	C	C	T	C	T
R. menchuae	A	C	A	C	T	T	C	T	T	T	T	T/C	C	C	A/G	C	C	T	C	T
R. tumida	A	C	A	C	T	T	C	C/T	T	C	T	T	C	T	A	C	C	T	C	T
R. velilla	A	T	A	T	T	C	T	T	C	C	T	C	C	C	A	C	C	C	T	T
R. bickhami	A	T/C	A	T	T	C	C	C	T	C	C	T/C	T	C	A	C	C	C	C	C
R. genowaysi	G	C	G	C	C	C	T	C	T	C	T	C	C	C	G	C	C	T	T	C
R. io	A	T	A	T/C	T/C	T	C	T	T	C	T	C	C	C	A	T	T	T	C	C

	264	270	276	280	282	285	291	300	303	312	318	321	324	333	336	342	348	351	354	355
R. aeneus	T/C	T	C	C	A	C/T	T	A	C	T	T/C	T	T	A	T	T	C	T	C	C
R. menchuae	T	T	C	C	A	C	T	G	C	T	C	T	T	A	T	T	C	T	C/T	T
R. tumida	T/C	T	C	C	A	T	T	G	C	T	C	C	T	A	T	T	C	T	C	T
R. velilla	C	C	C	C	G	T	T	A	C	C	C	C	T	A	T	C	T	T	C	C
R. bickhami	C	T	C	C	A	C/T	T	G	C	T	C	T	T	A	T	C	T/C	C	C	C
R. genowaysi	C	T	T	T	A	T	C	G	C	C	T	T	T	G	C	C	C	T	T	T
R. io	C	C/T	T	C	A	C	T	G	T	T	C	T	C	A	T	C	T	T	T	T/C

APPENDIX II (CONT.)

	358	363	366	369	384	387	390	393	396	399	402	417	420	426	438	447	448	459	462	465
R. aeneus	C/T	T	T	T	C	A/G	T	T	A	C/T	A	A	T	A	T	G	T	C/T	A	C
R. menchuae	C	T	T	T	C	G	T	T	A	C/T	A	A	T	G	C	A/G	T	C	A	T
R. tumida	T	T	T	T	C	G	T	T	A	C	A	A	T	G	T	G	T	T	G/A	T
R. velilla	C	C	C	T	C	G	T	C	A	C	A	A	T	G	T	A/G	C	C	A	T
R. bickhami	C	T	C	T	C	G	G/T	C	G	C	A	A	T	A	T	A/G	T	C/T	A	T
R. genowaysi	C	C	C	C	C	A	A	C	A	A	G	A	C	A	T	A/G	T	T	A	C
R. io	C/T	C	C	T	T	A	A	T	A	T	A	T	C	A/T	T	C/A	T	C	A	T

	471	474	475	480	483	498	525	537	540	546	550	555	561	567	570	573	585	588	597	600
R. aeneus	A	C	A	T	A	A	C	T	C/T	C	C/T	T	C/T	T	A	C	C	C	C	A
R. menchuae	A	T	A	T	A	A	C	T	C	C	C	T	C	T	A	C	C	C	C	A/G
R. tumida	A	A	A	T	A	A/G	C	T	T	C	C	T	C	T	A	C	C	C	C	A
R. velilla	T	T	A	T	A	A	G	T	C	T	G	C	C	C	C	C	T	C	C	A
R. bickhami	A	A/G	G	T/C	A	G	A	T	C	T	T	C	T	T	A	C	C	C	C/T	A
R. genowaysi	A	A	G	C	A	G	A	C	C	T	C	C	C	T	A	C	C	T	T	G
R. io	A	C	A	T	G	A/G	A	C	C	C	C/T	C	C	C	A	T	T	C	C	A/G

	606	612	615	618	624	627	630	633	639	648	651	657	660	666	669	672	681	684	687	690
R. aeneus	A	C	C	C	A	A	C/T	C	C	T/C	A	C	T	T	C/T	C	A	T	T	A
R. menchuae	A	C	C	C	A	A	C	C	C	T	A	C	T	T	T	C	A	C	C/T	A
R. tumida	A	C	C	C	A	A	C	C	C	T	A	C	T	T	T	T	A	C	T	A
R. velilla	A	A	C	C	A	A	C	T	T	C	A	C	T	T	T	T	A	C	C	A
R. bickhami	A	A	C/T	C	A	A/G	C/T	T	T	C	G	T/C	T	T	C	T	G	C	T	A
R. genowaysi	G	A	C	T	A	G	C	T	T	T	A	T	T	C	T	C	A	C	C	G
R. io	A	A	A/C	C	C/T	A	T	C	T	T/C	A	T/C	C	T	T	C/T	A	C	C/T	A

APPENDIX II (CONT.)

	696	699	702	706	708	712	713	714	718	722	729	732	735	738	744	748	753	756	759	762
R. aeneus	A	T/C	G	G	C	A/G	C	C	T/C	C	A	A	C	C/T	C	T	A	C/T	C	T
R. menchuae	A	T	A	A	C	G	C	C	T	C	A	A	C	C	T	T	A	T	T	T/C
R. tumida	A	T	A	G	C	G	C	C	T	C	A	A	C	C	C	C	A	C	C	T/C
R. velilla	G	T	A	A	T	G	T	C	T	C	C	A	C	T	C	C	A	C	C	C
R. bickhami	A	T/C	A	A	C/T	G	T	C	T	T	T	A	T	C	T	T	A	C	C	T
R. genowaysi	A	T	G	A	T	A	T	A	C	C	C	A	T	T	T	C	T	C	C	T
R. io	A	C	A/G	A/G	T	G	C/T	T	T	T	T	G	C	T	T/C	T	A	C	C	T

	765	768	771	774	783	784	789	798	807	816	819	822	831	846	852	858	861	864	867	873
R. aeneus	T/C	T	A	A	A	C	C	T	A	A	T/C	T/C	A	A/G	T	T	A	G	C	C
R. menchuae	C	T	A	G	A	C	C	T	A	A	C	T	A	A	T	T	A	G	C	C
R. tumida	C	T/C	A	A	A	C	C	T	A	A	C	T	A	G	T	T	A	G	T	T
R. velilla	C	C	G	A	A	T	T	C	A	A	T	T	G	G	C	T	A	G	T	C
R. bickhami	T	C	G	A	A	C	C/T	T	G	A	T/C	C	A	A	C	C	G	A/G	A/T	C
R. genowaysi	T	T	A	A	A	C	C	T	A	G	T	C	G	A	T	T	A	A	C	A
R. io	T	T	G/A	A	T	C	C	C	A	A	T/C	T/C	A	A/G	C	T	A	A/G	C	C

	876	885	888	891	894	897	901	906	912	915	921	924	927	930	933	945	947	951	957	960
R. aeneus	A	A/C/G	C	C	C	T	T	A	T/C	C	T	C/T	C	C/T	A	A	C	T	C	C
R. menchuae	A	G	C	C	C	T	T	A	T/C	C	T	C	C	C	A	G	C	T	C	C
R. tumida	A	G	C	C	C	T	T	A	C	C	T	T	C	T	A	G	C	T	C	C
R. velilla	A	A	C	C	C	T	T	A	T	C	C	C	C	C	G	A	C	C	C	C
R. bickhami	G	G/C	A	C	C	C	T	T	C/T	A	C	C	C	C/T	A/G	G	C	C	T	C
R. genowaysi	G	A	C	T	T	T	C	C	T	C	C	C	C	T	G	A	G	C	T	T
R. io	A/G	A	C	T	C	T	T	C	T	C/T	T	C	T	C/T	G	G	C	C	T	C

APPENDIX II (CONT.)

	966	970	972	975	979	981	981	990	993	996	997	999	1003	1029	1038	1041	1047	1050	1062	1065
R. aeneus	A	C	G	T	C	A	A	C/T	T	T/C	G	C	C	T	A	C	T	T	A	C
R. menchuae	G	C	G	T/C	C	A	A	T	T	T	G	C	T	T	A	C	T	T	A	A
R. tumida	A	C	G	T	C	A	A	T	T	C	G	C	T	T	A	C	T	T	A	C
R. velilla	A	T	A	C	T	A	A	C	C	T	A	T	T	T	A	T	T	T	A	C
R. bickhami	A	C	G	C	C/T	A	A	T	T	T	A/G	T	C	C	G/A	T	T	C	A	T/C
R. genowaysi	A	C	G	C	C	A	A	T	T	C	G	C	T	T	A	C	T	T	A	T/C
R. io	A	C	A/G	C	C	C	C	C	T	T	G	T/C	C/T	T	C	T	C	T	T/C	C

	1069	1071	1074	1083	1086	1089	1093	1095	1098	1104	1107	1110	1111	1122	1126	1128
R. aeneus	C	A	C	T	C	T	T	T/A	A	A	G	C	C	T	C	A
R. menchuae	C	A	C	T	C	T	T/C	A	A	A	G/A	C	C	T	C	A
R. tumida	C	A	C	T	C	T	T	A	A	A	G	C	C	T	C	A
R. velilla	T	A	C	T	T	C	C	G	A	A	A	C	C	C	C	G
R. bickhami	C	G	C	T	T	C	C	A	G	A	A	T	C	T/C	T	A
R. genowaysi	C	A	T	C	C	C	C	A	A	A	A	T	T	T	T	A
R. io	T/C	A	C	T	T/C	C/T	T	A	A	G	A	T	C	C	C	A